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Assessing the suitability of a manometric test system for determining the biodegradability of volatile hydrocarbons

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HIGHLIGHTS

- Abiotic losses of volatile hydrocarbons are significant in OxiTop© test systems.
- Biodegradation of volatile hydrocarbons, as measured by OxiTop©, are underestimated.
- Losses are attributed to sorption to plastic components of the test apparatus.
- Reduction or removal of plastic components improves the extent of biodegradation.

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ABSTRACT

Manometric test systems, adapted from those used to measure biochemical oxygen demand (BOD), and the OxiTop® test system in particular, are being increasingly used to determine the biodegradability of chemicals in accordance to OECD 301F guidelines. In this study, the suitability of the OxiTop® test system for determining the biodegradability of volatile hydrophobic substances has been explored. Experiments in biotic and abiotic systems were conducted with readily biodegradable complex aliphatic hydrocarbons covering a range of volatilities. Results indicated that abiotic losses of test substances were occurring due to sorption of the test substance to plastic components used in the OxiTop® system. A further 'plastic-free' biodegradation test system was designed using PreSens optical dissolved oxygen (DO) sensors. This significantly improved the measured biodegradation due to reduced abiotic losses and better retention of the test substance. These results highlight the importance of considering the physico-chemical properties of test substances when selecting test methods and equipment. They also highlight the value of incorporating chemical analysis and abiotic controls to improve the interpretation of biodegradation studies.

1. Introduction

Deliberate or accidental releases of organic pollutants into the environment are an unwanted side effect of the production and/or use of many products. The long-term impact of these pollutants is determined by the amount released, their fate and persistence in the environment, and their potential to cause harm to organisms. Various regulations have been developed to assess the impacts of organic pollutants in the environment. One such regulation is EU REACH (Registration, Evaluation, Authorization of CHemicals), which requires the evaluation of the biodegradability of high volume chemical substances produced by industry. Data from these evaluations are used in environmental exposure models to support risk assessment and for comparison to persistence criteria for the identification of priority PBT (persistent, bioaccumulative and toxic) substances. Under such regulations, biodegradation is often the key process used to assess the environmental persistence of organic chemicals.

Biodegradation, the catabolic breakdown of complex molecules by living organisms for energy and nutrients, is important for the elimination of a wide range of pollutants and wastes from the environment (Pagga, 1997). Microorganisms, mainly archaea,

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bacteria and fungi, are incredibly versatile in their ability to biodegrade a wide range of different types of organic pollutant. It is this versatility that allows the biodegradation of almost any organic molecule. In the case of hydrocarbons, this process works on the basis of sequential microbial oxidation, utilising oxygen to convert molecules over a series of reaction steps into CO_2 and H_2O (mineralization), plus other molecules that can be used in biosynthesis (Atlas, 1981; Rojo, 2009). The relative persistence of contaminants is influenced not only by their structural characteristics (Leahy and Colwell, 1990; Brown et al., 2017) but also their physicochemical properties and partitioning into different environmental media (soil, water, air), which can have a significant effect on their bioavailability to degrading organisms and thus the rate at which biodegradation occurs (Reid et al., 2000; Birch et al., 2017a, 2017b).

Assessment of biodegradability is usually performed experimentally using a tiered approach. The first tier (or screening level) involves a relatively cheap and stringent test of ready biodegradability corresponding to the OECD (Organisation for Economic Co-operation and Development) guideline no. 301 (OECD, 1992). This screens for those substances that are of no great concern in terms of environmental persistence and typically achieve >60–70% biodegradability within 28 days. The OECD 301 guideline provides a range of test methods (A to F) to suit the physical and chemical properties of the substance tested to maximize the chances of success.

Owing to their relative volatility and low water solubility, many hydrocarbons are tested using the OECD 301F Manometric Respirometry Test. Although it is recognised that problems may be encountered with more volatile components of test substances in the OECD 301F test, they may be assessed using this method provided precautions are taken (OECD, 1992). Furthermore, there is a range of equipment from several different suppliers that can be used to perform these tests (Battersby, 2000; Reuschenbach et al., 2003; Stasinakis et al., 2008) and their design can influence loss processes. In the process of investigating the capabilities of a number of contract research organisations (CROs) it was found that many were employing the OxiTop® kit manufactured by WTW (Weilheim, Germany) to conduct OECD 301F tests. This, like other manometric respirometry test systems, determines the oxygen consumption in closed respirometers via semi-continuous pressure measurements. Unlike some other test systems (ex. Co-Ordinating Environmental Services respirometer), the oxygen depleted during biodegradation is not replenished.

Several studies have used the OxiTop® system to determine hydrocarbon biodegradability but these have generally focused on heavier hydrocarbon products such as lubricating oils (Vähäoja et al., 2005a, 2005c). The suitability of the OxiTop® to determine the biodegradability of lighter, more volatile hydrocarbons has not been adequately demonstrated. Although the impact of their volatility on pressure readings is expected to be negligible, one of the main problems encountered when testing volatile hydrocarbons in other systems is that they can be lost from the test system through abiotic processes such as volatilisation and adsorption. This can lead to lower than expected BOD measurements and false negatives in ready tests, which could trigger further unnecessary and costly testing (Martin et al., 2017a, 2017b). It is therefore recommended that chemical analysis is employed to give an indication of abiotic losses that may have taken place (Whale et al., 2014).

This paper summarises an assessment of the suitability of the OxiTop® system for testing the biodegradability of a series of hydrocarbons with a range of volatilities. The test substances used are complex substances, consisting predominantly of n- and iso-paraffinic hydrocarbons produced by the Fischer-Tropsch catalytic process and have been previously demonstrated to be readily biodegradable in OECD 301F studies conducted to Good Laboratory Practice (GLP) standards (Best, 2014a, 2014b; Vryenhoef, 2014a, 2014b, 2014c, 2014d). In this work, additional chemical analysis was undertaken to assess abiotic losses, and a second test system based on optical dissolved oxygen (DO) sensors has been developed as a possible alternative to the standard OECD 301F test systems. Based on these investigations the potential shortcomings of the standard OxiTop® setup were explored and recommendations for improvement to tests systems to assess the biodegradation of volatile hydrophobic tests substances made.

2. Materials and methods

2.1. Chemicals

All reagents used in this work were purchased from Sigma Aldrich. The GTL hydrocarbon products used in this work were provided by Shell International BV. A summary of these products together with their relevant physico-chemical properties in shown in Supplementary Fig. S1.

2.2. Activated sludge

Activated sludge was collected from a facility in North Holland, The Netherlands that treats mainly municipal wastewater. The sludge was pre-conditioned to reduce endogenous respiration rate by twice centrifuging the sludge at 1000 g for 5 min and washing with mineral media. The sludge was then diluted to 1–2 g L^{-1} total suspended solids (TSS) with mineral media and aerated for 48 h prior to use to allow the removal of excess Biological Oxygen Demand (BOD) through microbial oxidation/reduction processes.

2.3. Mineral media

The mineral media used in this study was that recommended by the OECD 301 guidelines (OECD, 1992). Briefly, it consisted of 0.6 mM KH_2PO4, 1.25 mM K_2HPO4, 1.1 mM $Na_2HPO_4 \cdot 2H_2O$, 0.1 mM NH_4Cl, 0.2 mM $CaCl_2$, 92 μM $MgSO_4 \cdot 7H_2O$ and 0.9 μM $FeCl_3 \cdot 6H_2O$, 0.043 mM N-allylthiourea, pH 7.4.

2.4. Setup of the OxiTop® system

Each biodegradation vessel was setup as follows. A predetermined volume of aerated and temperature equilibrated (21 °C ± 2 °C) mineral media, containing inoculum (15 or 30 mg L^{-1} depending on the experiment) was added to a test vessel containing a magnetic stirrer. Filled vessels were placed in the test incubator for 1 h and then removed in small batches (3–4) for substrate addition. The substrate was added volumetrically using a 10–20 μL glass syringe (see Supplementary Fig. S2 for dose rates). Once completed, the vessels were sealed with the manometric pressure indicator cap and placed back into the incubator. All experiments were performed at 21 °C with 180 rpm stirring. Biodegradation measurements were taken daily. All experiments were performed in duplicate unless otherwise stated.

2.5. Setup of PreSens oxygen sensor system

The system was set up per the manufacturer's instructions. Custom made, PTFE-lined PSt3 oxygen sensor spots (PreSens Precision Sensing, Germany) were attached to the inside of 600 mL Duran® bottles, approximately 15 cm below the bottle cap, with 2 μl of silicon-based glue and cured at 50 °C for 24 h. Sensor spots were calibrated using a 2-point (0% O_2 saturation and 100% O_2 saturation) calibration using nitrogen and air-filled bottles respectively.